PROJECT 1

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--PROJECT I    P. HAMOSH M.D.

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.
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NEW YORK, N.Y. 10022
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Application for Research Grant
(Use extra pages as needed)

Date: 6/30/75

1. Principal Investigator (give title and degrees):
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2. Institution & address:
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3. Department(s) where research will be done or collaboration provided:
Departments of Physiology, Anatomy, Pathology, the School Systems and the Medical Examiner

4. Short title of study:
SMOKING AND LUNG DEVELOPMENT: A PROGRAM PROJECT.
PROJECT #1. SMOKING AND FUNCTIONAL LUNG MATURATION.

5. Proposed starting date: 1/1/76

6. Estimated time to complete: 5 years

7. Brief description of specific research aims:
This project is a composite of four studies: A. The Effect of Smoking on Maximum Expiratory Flow (MEF) in Teenagers. This study is designed to answer the following questions: 1) Do changes in MEF effected by smoking differ according to sex, race and environmental factors (coincident air pollution and economic factors)? 2) Do changes in MEF over the years of development depend on the intrinsic pattern of MEF ( in other words, does one pattern of MEF lead to chronic changes upon smoking, while another does not)? The study will be conducted in the Washington Metropolitan area school district in the form of annual lung functions and questionnaires in several schools located in different areas; providing therefore a diverse ethnic, environmental and socio-economic data base. B. Smoking and "small airway" Disease A Study in Pathology. A study designed to correlate smoking history, post-mortem lung functions and lung pathology in young victims of sudden death. This study will be in co-operation with the Medical Examiner. It will test the hypothesis, that those subjects having pathology associated with smoking have different airway patterns than subjects who have no pathology. This study provides the morphological basis for study A. C. Mechanical Stresses and Lung Pathology. This study will attempt to answer the question: Do mechanical forces contribute significantly to the deformation of airways and lung parenchyma and is the degree of damage related to intrinsic variation in lung structure? This will be a study in animal models, where structural changes will be induced by surgical manipulation or proteolytic enzymes and then the animals with altered lungs exposed to mechanical lung stress. Morphometry of these lungs, when compared to controls will provide the answer. D. The Effect of Smoking a Single Cigarette on the "small airway". This study is essentially a continuation of the study presently supported by the CTR (#878). It is an attempt to separate susceptible from nonsusceptible subject by "provocation"



1003545642

test, i.e. acute exposure. The question asked is: 1) How is the response affected by ethnic and racial background. 2) alteration of smoke (e.g. filters) and 3) pretreatment by pharmacological agents? These questions are answered by sophisticated plethysmographic studies before, during and after smoking of Kentucky brand cigarettes by volunteers.

P. KAMOSH M.D.

Project 1: SMOKING AND FUNCTIONAL LUNG MATURATION

A. The Effect of smoking on Maximum Expiratory Flow (MEF) in Teenagers

BACKGROUND:

Adult smokers can be separated with different degrees of success from non-smokers on the basis of lung function tests, depending on the sophistication of the test and the population studied (1). Most extensive studies were done in white subjects (2). Recent studies indicate that such separation is impossible in blacks and orientals (3). Less is known about lung functions in smoking teenagers.

Cigarette smoking was linked to pathogenesis of heart and lung disease (1). Whereas smoking a cigarette produces immediate changes in airway conductance and heart rate (2), it is less clear what the long term effects are. All smokers develop lung function changes (3), however, it is not clear whether these changes reflect the fact that a person is a smoker, or are only indicators of early lung disease. Many smokers develop chronic lung disease, but the vast majority do not. The question is: are these latter smokers more "protected" or "less susceptible"? No tests are presently available that separate "less susceptible" or "protected" individuals from "susceptible", in spite of major efforts by many groups, including ours.

Young smokers have early functional abnormalities (4), some of which are reversible after discontinuation of smoking (5,6). Sometimes these functional abnormalities are accompanied by respiratory symptoms such as cough, phlegm production, etc. (7). The prevalence of respiratory symptoms in adolescents, (11 years and older) smoking more than 5 cigarettes a day approaches the one reported for adult smokers (7). There is a correlation between smoking habits and respiratory symptoms which might suggest that chronic bronchitis starts early in life (7). Seely et al., (8) have reported significant changes in lung functions in smokers, age 15 to 19 years. They used the maximum expiratory flow volume (MEFV) curve (see figure 1). This test has been shown to be a sensitive detector of early functional changes, both in adults (9,10) and children (11). The incidence of respiratory symptoms increased as cigarette consumption increased. More smokers than non-smokers had MEFV curves convex towards the volume axis. Flows at 50 and 25% of the vital capacity ($MEF_{50}$ and $MEF_{25}$) were significantly lower in those boys and girls smoking more than 15 and 10 cigarettes respectively (8). One out of five teenage smokers had abnormal lung functions not detected by conventional spirometry (12).



1003545644

In rapidly growing children, lung size increases faster than total body size (13). Therefore, the "growth" of the MEFV curve is related to total lung capacity. Most normal children have MEFV curves that are concave towards the volume axis . It was suggested that "damage" caused by cigarette smoking at an early age may affect lung development (14). On the other hand, Green et al. (15) suggested that basic morphologic reapportion between lung parenchyma and airways may be a factor in the pathogenesis of disease, preceding exposure to any noxious agents. Certain patterns of lung structure may predispose to lung disease when associated with cigarette smoking. They introduced the word "dysanaptic" for lungs that do not develop isotropically Damage to the lung when still growing could further disturb the airway parenchyma relationship. Recent work by Reid et al. (16,17) suggests that abnormal subdivision of the bronchi might be one of the causes of emphysema, a concept recently amplified by Kilbourne (18). Zapletal et al. (19) suggested that one of the abnormalities of cystic fibrosis is a "maturation arrest" of the lung.

We assume then, as a working hypothesis, that hereditary, congenital or acquired (early in childhood) anatomical changes might produce a respiratory pump which is predisposed, (by virtue of its mechanical properties) to chronic obstructive lung disease (COLD) when associated with other environmental factors, such as smoking, air pollution and infection.

PRINCIPAL AIMS:

1. Study the evolution of the maximum expiratory flow volume curve (MEFV) in children and adolescents.
2. Correlate history of early lung disease and smoking habits with the evolution of the MEFV curve, to identify the type of curve associated with susceptibility to COLD.
3. Analyze these data also, in relation to sex, ethnic background, economic factors, air pollution, etc.

EXPERIMENTAL DESIGN:

To realize the above aims, a prospective study utilizing school age children will be initiated with the following principles in mind:

1. A wide data base, to assure continuity
2. Simple and non-invasive tests, to assure child and parent cooperation
3. Minimum disruption of classrooms, to assure cooperation of school authorities.

4. A modern data acquisition storage and retrieval system, to assure rapid processing of information.

MATERIALS AND METHODS:

The subjects will be school children between the ages of 11 to 18. A number of elementary, junior high and high schools will be selected to represent an ethnic and socio-economical cross section of the Metropolitan Washington area. We will aim for 1,300 students annually, considering the demographic realities and frequency of migration (See epidemological and statistical consideration).

MODE OF OPERATION:

The parents will receive a detailed explanation of the purpose and scope of the study signed by the school principal. The parent will return a signed consent form and answer a few simple questions such as: the parent's smoking habits, family and child's history of lung disease, possible exposure to pollutants, etc. The child will then proceed with this consent form to the testing station in the school where he will answer a few questions regarding smoking history. His vital statistics will be taken and spirometry performed. The data will be processed at the processing station at the University and then stored for easy retrieval.

DATA ACQUISITION:

1. Questionnaires (Samples appended).. The design is conducive to simplicity and coding for punch cards.

2. Spirometry Three consecutive maximum expiratory efforts will be performed by the child through a pneumotachograph. Flow will be integrated into volume and the two analog signals (flow and volume) digitized on line and recorded by a digital cassette recorder. The analog signal will also be displayed in real time on a storage oscilloscope for monitoring purposes.

DATA PROCESSING:

A. Theoretically Maximum expiratory flow (MEF) is effort independent below 70% of vital capacity and therefore an ideal, highly repeatable measurement of

mechanical lung function (20). The magnitude of MEF at any given lung volume is determined by the elastic recoil of the lung ($P_L$) and the resistance of the "upstream" segment of airways ($R_{us}$) called so because they are upstream from the point where airflow limitation occurs (21). Since early changes in COLD are probably in the "small airway" (those less than 2mm in diameter), early changes in MEF at low lung volume would be expected (22). If there are developmental and/or maturational abnormalities or variants in airway anatomy they are more likely to be in the small airways and therefore cause a change in the $R_{us}$ and consequently in MEF at low lung volumes, in other words, the mid or terminal part of MEF is probably the measurement worth the maximum attention when looking for subtle changes in small airway function (See also progress report of Grant # 878).

B. PRACTICAL MEASUREMENTS: (Read with accompanying diagrams)

Maximum expiratory flow will be performed through a disposable mouthpiece connected to a corrugated tube leading into a #3 Fleish pneumotachograph (PNTG). The pressure drop across the PNTG will be picked up by a differential pressure transducer (Validyne PM 46).and amplified by a carrier amplifier. This flow signal will then be integrated by an integrator.(This whole apparatus will be purchased from Hewlett-Packard, who have developed a compact package. However, at the time of writing this proposal, we are evaluating two more systems). The output is two analog signals: flow and volume. These could be recorded by digital or FM analog tape recorder. We are in the process of evaluating both methods to arrive at a simple and inexpensive solution. To minimize cost we are trying to develop a system which can be interphased by existing computational facilities so that the great volume of information could be rapidly processed. During the actual measurements under "field conditions" the flow and volume signals will be displayed on a storage oscilloscope for immediate evaluation of the correctness of procedure and quality of tracing. The signals will be A/D converted or directly taped at the "field station." The record consists of a segment on a magnetic tape and the questionnaires.

C. PROCESSING AT "HOME STATION"

The following derived parameters will be obtained by digital computer:
Observed and Predicted Values for:

1. Forced Vital Capacity (FVC)

P. HAMOSH M.D.

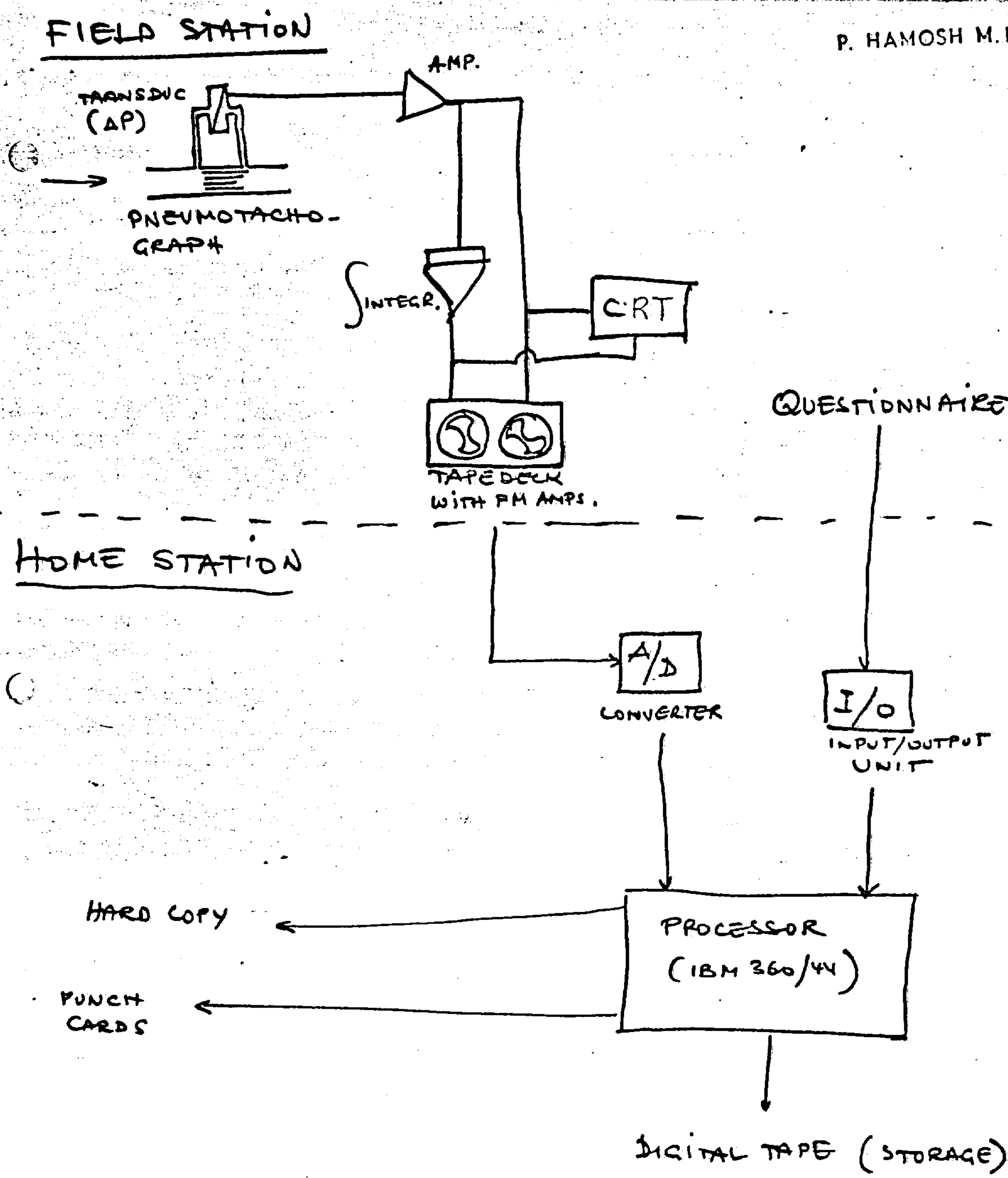


FIGURE 5.

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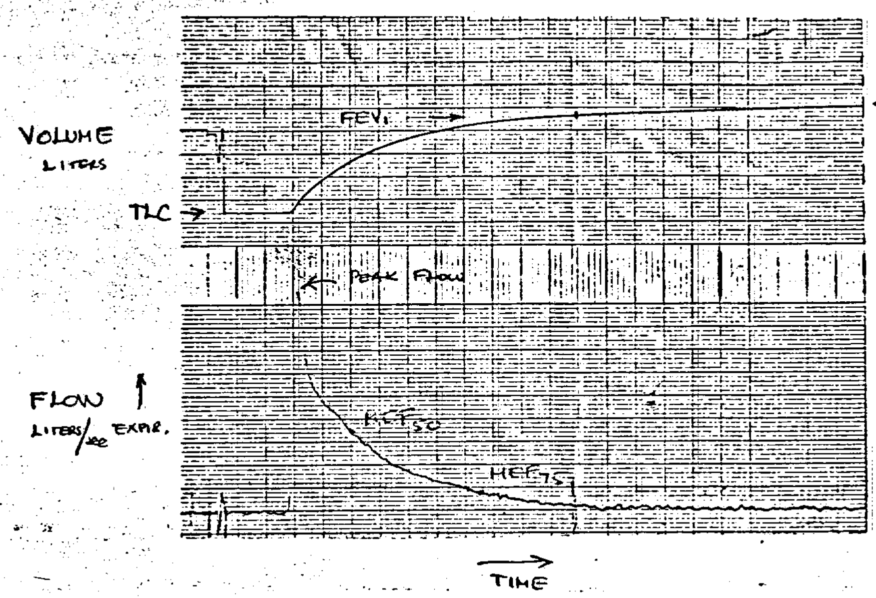
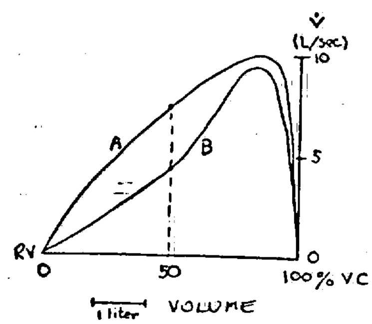
## EXPIRATORY SPIROGRAM




SIGNALS AQUIRED
↓
INTEGRATED
↓
RECORDED ON TAPE

## MAXIMUM EXPIRATORY FLOW-VOLUME curve

↓
DISPLAY




EXPIR.
FLOW

A represent the MEFV curve as seen in young adults
B is more characteristic of adult smokers

FIGURE 6.



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2. Forced Expiratory Volume (FEV) at any lapse of time
3. $FEV_1$ to FVC ratio
4. Instantaneous Flow at any point of vital capacity ($MEF_x$)
5. Maximum Mid-Expiratory Flow Rate (MMFR)
6. Terminal Flow Rate ($MTF_{50-75}VC$)
7. Peak Flow
8. Momentum Analysis of the Expiratory Curve.

D. EPIDEMIOLOGICAL andSTATISTICAL CONSIDERATIONS :

A recent workshop conducted by the National Heart and Lung Institute (NHLI) on the early detection of lung disease deals at length with the experimental design of epidemiological studies of this nature (23). We consider this study primarily a study of structure and function; however, it would be foolhardy to ignore the epidemiologic implications. Therefore we decided to select the number of our subjects and the demographic composition of the group on the basis of valid statistical criteria. Our preliminary estimate is 1,300 subjects, but at the time of writing this proposal, not all the necessary demographic information was available, for the exact calculations. See the separately submitted addendum.

NOTE: At the writing of this proposal, we are seeking to concert our efforts with other groups planning to engage in similar prospective studies, provided we can agree on standardizing our equipment. This study might become a part of a co-operative effort.If an accord is reached before September, an addendum to this proposal will be forthcoming.

P. HAMOSH M.D.

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P. HAMOSH M.D.

PROJECT 1B

Smoking and "small airway" disease, a pathological study.

BACKGROUND:

It has been suggested that chronic obstructive lung disease initially affects the airways less than 2mm in diameters, designated as "small airways" (1). Pathological studies in young smokers who died suddenly showed respiratory bronchiolitis (2). Morphometric studies showed higher incidence of emphysema in young smokers (3) but the airways received less attention. Kleinerman et al.(4) did perform studies on postmortem lungs, which showed a lesser evidence of small airway involvement than suggested by Hogg et al.(1). What is missing to this day is a comprehensive study of physiology, morphology and smoking history methodically performed on a sufficient number of post-morten lungs, to yield statistically meaningful data. The question to answer is: what is the nature and distribution of lesions in the lungs of young people, smokers and non-smokers and how does it correlate to life history and ante or post-mortem lung function? The aim of this study will be to accumulate over several years about fifty lungs from young subjects, victims of sudden death, with known history of illnesses and smoking habits; perform pulmonary functions on the isolated lung and then prepare them for histology by pressure fixation. Morphometry will then be performed by automated light microscopy and scanning electron microscopy.

EXPERIMENTAL DESIGN:

The subjects will be selected from the Medical Examiner's cases, preferably subjects who died instantly from head injury, without damage to the chest. If both lungs cannot be obtained at least one whole lung will be used. The subject qualified for study if he has family available for history at a later time, the lung is not leaky and the post-mortem is performed within 24 hours of death.

Pressure volume flow relationship will be studied on the isolated lung (see Methods) which will then be pressure fixed. Subsequently one large sagittal section will be made for mounting on paper. Blocks for morphometry will be selected by random approach (see Methods) and slides prepared for light microscopy and scanning electron microscopy. Morphometry

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will be automated by using pattern recognition computer technology.

METHODS:

1. Lung Mechanics

The isolated lung will be connected by the airway to a pneumotachograph and suspended in a volume plethysmograph. Flow will be measured by the pneumotachograph - differential pressure transducer system, the volume changes by a Krogh spirometer, and transpulmonic pressure by a transducer connected to the airway orifice. All signals will be amplified by carrier amplifiers and displayed on either direct writing recorders or oscilloscopes. The experimental apparatus is shown in Figure 7. A total analysis of volume/pressure (compliance), volume/flow and flow/pressure (conductance) relationships will be measured. Total respiratory resistance and dynamic compliance will be measured also as a function of frequency. For this end, the lung will be oscillated by a high power speaker.

Morphometric Studies:

On completion of the physiological studies, Barium Sulfate dust (Micropaque, Nicholas Laboratories) will be insufflated into the bronchial tree and roentgenograms will be taken at TLC (Total Lung Capacity). The transverse diameter of each segmental bronchus will be measured at a point 0.5 cm from its bifurcation and corrected for magnification. The lungs will be inflated and fixed in 20% neutral buffered formalin in 72 hours at 25 $cmH_2O$ pressure. The lung will be cut into 1 cm parasagittal slices and carefully examined. The amount of emphysema, if present, is determined by point count (6). Ten randomly selected 2 x 2.5 cm sections will be cut from each lobe using a metal template. Transverse sections will be taken from the upper and lower lobe bronchi. The sections will be embedded in paraffin and 6 $\mu m$ sections will be stained with H & E. Parenchymal sections with significant artifact will be excluded. Six of the remaining sections from each lobe, a total of 60 $cm^2$ fixed tissue, will then be randomly selected for study. The number and size of the non-cartilagenous membranous bronchioles less than 2 mm internal diameter will be evaluated using the method of Matsuba and Thurlbeck (7). First the bronchiole density will be determined and expressed as bronchioles/$cm^2$ fixed tissue. A bronchiole will be counted only if at least 75% of its perimeter clearly consists of membranous bronchiolar wall. This excludes some airways at the edge of sections and some of those cut obliquely

through the junction of the terminal and respiratory bronchioles. Next, the diameter of each bronchiole (the longest distance in the short plane) will be measured and ranked by size in 50 μm increments. Because the epithelium will be desquamated in some of the lungs, measurement in all cases will be made from basement membrane to basement membrane.

Automated Morphometry

Morphometry of the lung has been greatly improved and standardized in recent years through the work of Weibel, Thurlbeck and Reid (8), but it still represents a very time consuming task. The standard method based on the mathematical work of Tomkieief (9) and Delgado (10) is known as the "point counting" method. A grid of points is superimposed on the microscopic image and the elements coinciding with the point are counted. Needless to say, the number of points is limited and the observer can just count a short time. Because of fatigue, all studies of this nature have to be done double blind, to eliminate bias by the counter.

Scanners have been introduced years age into morphometric work. They have cut the time of counting, however, could not recognize cellular elements of tissue patterns, therefore were limited for use in tasks where no judgement was needed. The next big step ahead was the development of computerized image analysis known as "pattern recognition."

The National Biomedical Research Foundation (NBRF) directed by Dr. Ledley is equipped with a 360/44 IBM computer with all the peripherals to do automated morphometry in this system. The system is described in the appendix. We can count alveoli, bronchi, arteries and other elements. Our own contribution is appended (Ungerleider and Hamosh). This service will be provided to projects 1B, 1C and 3. The personnel of NBRF will also assist in the development of hardware and software for 1A. (See appendix, especially the description of the SPIDAC System).

P. HAMOSH M.D.


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9. See addendum to follow

10. See addendum to follow

P. HAMOSH M.D.

PROJECT 1C

THE EFFECT OF MECHANICAL STRESSES ON LUNG TISSUE.

BACKGROUND:

We have suggested the possibility that shearing stresses in the airways might be an important contributing factor in the pathogenesis of chronic obstructive lung disease or even carcinogenesis (1). Shearing forces generated during maximum expiratory flow (MEF) and particularly during coughing might produce significant damage to bronchial mucosa. (see appended manuscript draft). This damage would be particularly great in the area where sudden narrowing of airways occurs. Such an area exists just downstream from the equal pressure point (EPP). Constant degeneration and regeneration of the bronchial epithelium in this area might lead to metaplasia and ultimately malignant changes. Is it a coincidence that bronchogenic carcinoma arises mostly from the third or fourth division of the bronchi (2), the usual site for the EPP during coughing and MEF from high lung volume? The "yield stress", the level of shearing forces necessary to induce irreversible epithelial changes might be reduced when other factors weaken the epithelium. Such factors could be proteolytic enzyme, local edema or inflammation produced by chemical, immunological or infective agents, etc. Under these conditions, the small airway could also become a site of damaging shearing stresses.

Simple "perpendicular" stresses also become a factor as we approach the terminal lung units. These stresses produce strain on the tensile elements of lung parenchyma and these are well defined and limited (3). If increased mechanical wear and tear is combined with structural weakening, the tissue will yield, i.e.: there will be alveolar septal disruption. Translating this to a human example: given two subjects with the same structural weaknesses in the lung (e.g. $alpha_1$ antitrypsin deficiency) the subject exposed to mechanical stresses of the lung (heavy laborer, smoker with cough) will develop emphysema more rapidly. Our working hypothesis is: mechanical stresses and structural weakness are additive in nature, and both shearing and perpendicular stresses could lead to irreversible lung damage conducive to chronic obstructive lung disease and carcinogenesis.

The evidence so far is slim, since very little work has been performed in this area. We showed that shearing stress can indeed disrupt isolated tracheal epithelium (1 and appendix). We also made attempts by exposing rats to papain and have half of them swim for two hours a day for one week after papain exposure. The differences in lung structure and function were not significant. We fault the experimental design because the effort of swimming was insufficient. The experimental design we expect to give results is described below:

This hypothesis raises the interesting possibility that cough induced by irritants might be more responsible than the irritant itself for the effects. How does this tie in with lung development? If anisotropic lung growth or dysanapsis (4) on the one hand and developmental arrest, such as suggested after bronchiolitis (5) or cystic fibrosis (6) on the other, lead to the development of smaller airways, the effect of shearing stress will be magnified exponentially. The consequences of perpendicular stresses will occur earlier in a subject who has structural weakness (increased proteolysis, connective tissue displasia, etc.) and may even lead to maturation slowdown, which in turn yields "dysanaptic" lung and the vicious circle is closed.

PRINCIPAL AIMS

The principal aims of this study are:

1. To establish the "yield" stress of lung tissue in vivo to perpendicular and shearing stress.

2. To produce models of decreased "yield" stress by adding proteolysis to stresses.

3. To attempt to correlate sites of maximum "yield" to maximum damage in the animal and human.

EXPERIMENTAL DESIGN - GENERAL

1. The effect of shearing stress

a. Rats and hamsters will be used in these experiments. Tracheal stenosis will be produced in rats by creating a sudden stepwise narrowing by surgical procedure. The animals will be sacrificed at set time intervals and the transitional area examined for damage under light and scanning electron microscopy (see appended manuscript for methodology).

P. HAMOSH M.D.

b. Homotransplants of hamster trachea (7) will be stressed chronically and then inspected for epithelial damage.

c. Human postmortem material will be examined for coincidence of epithelial damage with high shear distribution (see also Project #1B).

2. The effect of perpendicular stress

Assuming that hyperventilation of exercise is the most natural model for increased mechanical lung stress, rats or hamsters will be exposed to a proteolytic enzyme and then divided into a control group and an exercise group, using a treadmill. Alternative models would be total body restraint (8) or the "whirling" mice (9). After a suitable period, the animals will be sacrificed and lung mechanical properties and morphometry compared. The developmental aspect will be explored by using animals at different stages of their growth.

Project 1/B

METHODS:

a. Shearing Effects (see appended manuscript)

The airways exposed to shearing stress will be excised and mounted in longitudinal strips. Part of this strip will be fixed for longitudinal sectioning and staining and other parts will be prepared for scanning electron microscopy. The damage produced by shearing stress will be graded by the method introduced by us (10). Local shearing stresses will be calculated by exposing plastic casts of the stressed airway to in vivo flow rate and measure pressure drops. These methods are described in some detail in the appended manuscript. Results will be interpreted by correlating local shearing stress to epithelial damage.

b. Perpendicular Mechanical Stress (See flowsheet)

The effects of perpendicular stress are best studied by correlating the amount of hyperventilation to damage of lung structure as determined by morphometry.

1. Method of Stressing:

Hamsters will be exercised on a Quinnton rodent treadmill once a day for two hours and four hours respectively. Half of the hamsters will be pretreated by collagenase and elastase administered by nebulization. Stressing will only be done after recovery from initial acute damage. The methodology was described (11).

2. Measurement of Elastic Properties and Tensile Strength

Upon sacrifice of the animals the lung will be removed in toto and the volume-pressure relationship measured by the static step by step deflation method (12). The tensile strength will be measured by the method of C. J. Martin (13).

3. Evaluation of Damage

Morphometric analysis will be made of the pressure fixed lungs (14). The internal surface area (15) and the pattern of destruction of alveolar septae (16) will be studied (see section on morphometry).

Significance

If shearing or perpendicular stresses do contribute significantly to disruption of airways and alveoli, they probably represent a significant additive factor in the pathogenesis of COLD. It is not now known how cough or hyperventilation affect lung structure. Since clarification of this problem might influence the management of COLD, this is also a clinically important study. The developmental aspect of the problem is in the study of the "yield stress" as a function of age. Is the developing lung more or maybe less susceptible to damage from mechanical stress ? While some information exists about the composition of the lung as a function of age (17), very little is known about tensile strength of the lung (18) as a function of age.

P. HAMOSH M.D.

| Group A | Group B | Group C | Group D |
|---|---|---|---|
| Nonexercised (Care) Controls | Exercised Controls | Exercised Emphysematous | Nonexercised Emphysematous |

↓

Lung Mechanics + Tensile Strength

↓

Pressure Fixation

↓

Embeding, Sectioning, Mounting and Staining

↓

Morphometry for internal surface area (ISA)
and patterns of lung destruction

↓

Analysis of Data: Correlation between stress, emphysema, the compliance curve, lung recoil, tensile strength and the magnitude and pattern of lung damage

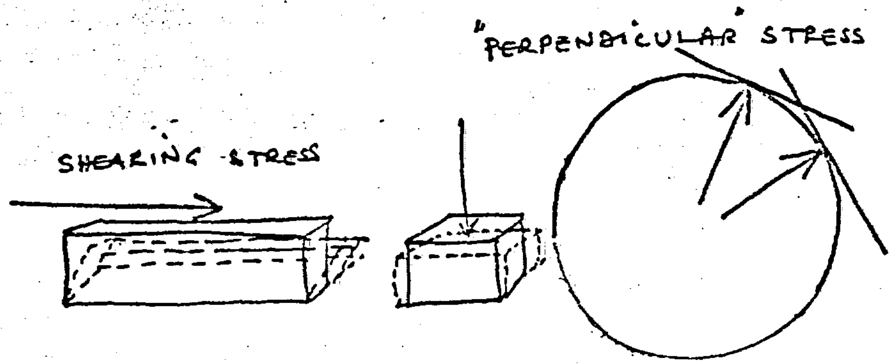


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FIGURE 8.

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P. HAMOSH M.D.

PROJECT 1/D

The effect of smoking a single cigarette on the "small airways".

BACKGROUND:

This is a continuation of our research on this subject (1). We have now established that the response is immediate (plateaus after three puffs); it is not dose dependent, but it is cigarette dependent (Kentucky brand 1R1 shows a more profound effect than brand IA1); it probably exerts first effect on the large airways (airway resistance) and the effect on "small airway" (midexpiratory flow) is delayed and also slower to return to normal after cessation of smoking (2).

We still have not resolved whether a "lung mechanics" test will be able to separate "reactors from non-reactors". Presently we are engaged in the study of ventilation distribution as a measure of single cigarette effect. Our results on the effect of a single cigarette were corroborated by Bouhuys et al (3), and are frequently cited in connection with maximum expiratory flow at 50% vital capacity ($MEF_{50}$) as the most valuable single measurement in separating chronic smokers from non-smokers (4).

AIMS:

We would like to pursue this research for several more years to answer the following questions:

1. Response to the same cigarettes we studied (1r1,1a1 and 1a3 smoked through a Cambridge filter.
2. Response to smoke other than cigarettes
3. Response to cigar or pipe smoke
4. Pretreatment with drugs and modification of response.

EXPERIMENTAL DESIGN:

Young volunteers, mostly students and faculty will smoke a cigarette in a body plethysomograph and "small airway" functions will be measured by the methods described in detail in the appended proposals, progress reports and publications (Ref. 1,2,4, Proposal 1A and progress report of CTR grant #878R2).

References:

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First Year Budget
Project 1.

P. HAMOSH M.D.

3.

13. Budget: (1st year)

| | % time | Amount |
|---|---|---|
| **A. Salaries (Personnel by names)** | | |
| Professional * | | |
| Angelo M. Traveira Da Silva (A) | 50% | 14,794 |
| Technical* Linda Cooper (A) | 100% | 11,816 |
| To Be Hired (Histology) (Bec) | 100% | 11,053 |
| To Be Hired (Machine) (A&D) | 100% | 10,068 |
| * All Salaries Include 18.35% Fringe Benefits (16.7% 1/1/76-6/30/76 and 20% 7/1/76-12/31/76) | Sub-Total | 47,731 |
| **B. Consumable Supplies (list by categories)** | | |
| Chemicals, Disposables | | 3,000 |
| Histology, Microscopy | | 2,500 |
| Photographic, Recording | | 2,000 |
| Animals and Care | | 2,500 |
| | Sub-Total | 10,000 |
| **C. Other Expenses (itemize)** | | |
| Computer Time (A,B) | 100 hours | 9,000 |
| Travel | | 2,000 |
| | Sub-Total | 11,000 |
| **D. Permanent Equipment (itemize)** | | |
| Computer System "Field Station" And Interface (A) | | 15,000 |
| Custom Made System For Pressure Fixation (B) | | 1,500 |
| Surgical Equipment (B&C) | | ~~900~~ |
| Custom Made Plethysmographs (B&C) | | 2,000 |
| | Sub-Total | 19,400 |
| **E. Overhead (15% of A+B+C)** | | |
| | Total | 88,131 |

ok

Overhead to be negotiated at 15% or higher of A,B, & C

The Distribution of efforts by all investigators is presented in Figure 2

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